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SWANSON & BRATSCHUN L.L.C.
1745 SHEA CENTER DRIVE
SUITE 330
HIGHLANDS RANCH, CO 80129

| EXAMINER | |
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| STRZELECKA, TERESA E | |
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Please find below and/or attached an Office communication concerning this application or proceeding.

PTO-90C (Rev. 07-01)

| **Office Action Summary** | **Application No.** 09/864,637 | **Applicant(s)** WEI ET AL. |
|---|---|---|
| | **Examiner** Teresa E Strzelecka | **Art Unit** 1637 |

*-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --*

**Peri d f r Reply**

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE 3 MONTH(S) FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If the period for reply specified above is less than thirty (30) days, a reply within the statutory minimum of thirty (30) days will be considered timely.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133).
- Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

**Status**

1)☒ Responsive to communication(s) filed on *05 May 2003*.

2a)☒ This action is **FINAL**. 2b)☐ This action is non-final.

3)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

**Disposition of Claims**

4)☒ Claim(s) *1-8,10-32 and 37-46* is/are pending in the application.

4a) Of the above claim(s) _____ is/are withdrawn from consideration.

5)☐ Claim(s) _____ is/are allowed.

6)☒ Claim(s) *1-8,10-32 and 37-46* is/are rejected.

7)☐ Claim(s) _____ is/are objected to.

8)☐ Claim(s) _____ are subject to restriction and/or election requirement.

**Application Papers**

9)☐ The specification is objected to by the Examiner.

10)☐ The drawing(s) filed on _____ is/are: a)☐ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

11)☐ The proposed drawing correction filed on _____ is: a)☐ approved b)☐ disapproved by the Examiner.

If approved, corrected drawings are required in reply to this Office action.

12)☐ The oath or declaration is objected to by the Examiner.

**Priority under 35 U.S.C. §§ 119 and 120**

13)☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

a)☐ All b)☐ Some * c)☐ None of:

1.☐ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____ .

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

14)☐ Acknowledgment is made of a claim for domestic priority under 35 U.S.C. § 119(e) (to a provisional application).

a) ☐ The translation of the foreign language provisional application has been received.

15)☐ Acknowledgment is made of a claim for domestic priority under 35 U.S.C. §§ 120 and/or 121.

**Attachment(s)**

1) ☒ Notice of References Cited (PTO-892)
2) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)
3) ☐ Information Disclosure Statement(s) (PTO-1449) Paper No(s) _____ .
4) ☐ Interview Summary (PTO-413) Paper No(s). _____ .
5) ☐ Notice of Informal Patent Application (PTO-152)
6) ☐ Other:

## DETAILED ACTION

1. This office action is in response to an amendment filed on May 5, 2003. Claims 1-36 were previously pending, with claims 33-36 withdrawn from consideration. Applicants cancelled claims 9 and 33-36, and added new claims 37-46. Claims 1-8, 10-32 and 37-46 are pending and will be examined.

2. Applicants' amendments overcame the following rejections: rejection of claims 10, 14, 15 (G-J), 24, 28, 31 and 32 (O) under 35 U.S.C. 112, second paragraph; rejection of claims 1-5, 7, 10, 11, 13, 14 and 32 under 35 U.S.C. 102(b) over Nelson et al.; rejection of claim 6 under 35 U.S.C. 103(a) over Nelson et al. and Somerville et al; rejection of claim 8 under 35 U.S.C. 103(a) over Nelson et al. and El-Meanway et al.; rejection of claims 9 and 12 under 35 U.S.C. 103(a) over Nelson et al. and Frohman et al. Applicants' arguments regarding previous rejections are moot in view of new grounds for rejection. However, since claim 9 has been incorporated into claim 1, arguments regarding this claim are presented below.

3. Applicants' amendments presented new grounds for rejection, therefore this office action is made final. The following are new grounds for rejection necessitated by amendment.

### *Claim Rejections - 35 USC § 112*

4. The following is a quotation of the second paragraph of 35 U.S.C. 112:

> The specification shall conclude with one or more claims particularly pointing out and distinctly claiming the subject matter which the applicant regards as his invention.

5. Claims 1-8, 10-32 and 37-46 are rejected under 35 U.S.C. 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention.

A) Claims 1-8, 10-14 and 38-41 are indefinite in claim 1, because claim 1 is indefinite over the recitation of the limitation "...<u>each individual member</u> of said non-normalized cDNA library is

hybridized to <u>a differential of the amount of labeled probe</u> of said labeled probe library" (lines 10-12 of the claim; emphasis added). It is not clear what is encompassed by the term "differential of a probe amount" and how it can be hybridized to a <u>single</u> member of the library. In particular, what does it mean for an amount of something to be differential? Does it mean a fraction of the amount, as opposed to the whole?

B) Claims 1-8, 10-14 and 38-41 are indefinite in claim 1, because the term "lower amounts of labeled probe" in claim 1 is a relative term which renders the claim indefinite. The term " lower amounts of labeled probe " is not defined by the claim, the specification does not provide a standard for ascertaining the requisite degree, and one of ordinary skill in the art would not be reasonably appraised of the scope of the invention. The specification does not contain a definition of what amount of labeled probe is considered "lower", and what it is lower with respect to.

C) Claims 15-31 and 43-45 are indefinite in claim 15, because the term "library is represented in lower amounts " in claim 15 (lines 10 and 26) is a relative term which renders the claim indefinite. The term " lower amounts " is not defined by the claim, the specification does not provide a standard for ascertaining the requisite degree, and one of ordinary skill in the art would not be reasonably appraised of the scope of the invention. The specification does not contain a definition of what amount of a library is considered "lower", and what it is lower with respect to.

D) Claims 15-31 and 43-45 are indefinite in claim 15, because the term "library is represented in higher amounts " in claim 15 (lines 12, 14, 23/24) is a relative term which renders the claim indefinite. The term " higher amounts " is not defined by the claim, the specification does not provide a standard for ascertaining the requisite degree, and one of ordinary skill in the art would not be reasonably appraised of the scope of the invention. The specification does not contain a definition of what amount of a library is considered "higher", and what it is higher with respect to.

E) Claim 30 is indefinite over the recitation of "said group of members" in line 4. Claim 30 is dependent from claim 15, where we have "one group of members" in line 10, "one or more groups of members" in line 11/12, "one or more groups of members" in line 14, "said group of members" in lines 16, 19 and 21/22. It is not clear to which group of members the term in claim 30 refers to.

F) The term "library represented in lower amounts " in claim 31 (line 3) is a relative term which renders the claim indefinite. The term " lower amounts " is not defined by the claim, the specification does not provide a standard for ascertaining the requisite degree, and one of ordinary skill in the art would not be reasonably appraised of the scope of the invention. The specification does not contain a definition of what amount of a library is considered "lower", and what it is lower with respect to.

G) Claims 32, 37 and 46 are indefinite in claim 32, because the term "library represented in lower amounts " in claim 32 (line 10) is a relative term which renders the claim indefinite. The term " lower amounts " is not defined by the claim, the specification does not provide a standard for ascertaining the requisite degree, and one of ordinary skill in the art would not be reasonably apprised of the scope of the invention. The specification does not contain a definition of what amount of a library is considered "lower", and what it is lower with respect to.

H) The term "library represented in higher amounts " in claim 43 (line 3) is a relative term which renders the claim indefinite. The term " higher amounts " is not defined by the claim, the specification does not provide a standard for ascertaining the requisite degree, and one of ordinary skill in the art would not be reasonably appraised of the scope of the invention. The specification does not contain a definition of what amount of a library is considered "higher", and what it is higher with respect to.

I) The term "library represented in lower amounts " in claim 44 (line 2) is a relative term which renders the claim indefinite. The term " lower amounts " is not defined by the claim, the specification does not provide a standard for ascertaining the requisite degree, and one of ordinary skill in the art would not be reasonably appraised of the scope of the invention. The specification does not contain a definition of what amount of a library is considered "lower", and what it is lower with respect to.

### *Claim Rejections - 35 USC § 103*

6. The following is a quotation of 35 U.S.C. 103(a) which forms the basis for all obviousness rejections set forth in this Office action:

> (a) A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negatived by the manner in which the invention was made.

7. Claims 1-5, 7 and 10-14 are rejected under 35 U.S.C. 103(a) as being unpatentable over Nelson et al. (Genetic Analysis: Biomolecular Engineering, vol. 15, pp. 209-215, 1999; cited in the IDS and in the previous office action) in view of Frohman et al. (PNAS USA, vol. 85, pp. 8998-9002, 1988; cited in the previous office action).

A) Regarding claim 1, Nelson et al. teach construction of normalized cDNA library. First, a non-normalized cDNA library was constructed from polyA+ RNA isolated from normal human prostate tissue. Members of the library were separated by plating individual clones onto 384-well microtiter plates, grown and replica-spotted onto nylon membranes. The replicas were allowed to grow on the membranes, and then the colonies were lysed, providing DNA bound to the membrane (page 210, third paragraph). A labeled probe library was constructed by reverse transcription of the polyA+ RNA isolated from normal human prostate tissue using oligo-dT-30 primer, reverse

transcriptase, dATP, dTTP, dCTP, dGTP and $^{32}P$-dCTP, in a reaction at 42 °C for 30 minutes (page 210, fourth paragraph).

The filters were hybridized with the labeled probe, exposed to phosphor capture screens and the signals were quantitated on a phosphorimager. Clones with signal intensities within the bottom quartile of the averaged intensities were selected (page 210, fifth and sixth paragraphs). A total of 842 cDNA clones were selected based on low hybridization intensities, of which 142 were discarded. Of the remaining 700 cDNA clones, 89% of sequences were genes of low abundance (= low expression) (page 211, fourth and fifth paragraphs; Table 1). The selected genes were pooled into a normalized library, PN001-NS (page 213, second paragraph; Table 1).

Regarding claims 2-5 and 7, Nelson et al. teach isolation of polyA+ RNA from normal human prostate cells. Nelson et al. do not specifically say that the RNA was mRNA, but only mRNA in the eucaryotic cells is polyadenylated (page 210, third paragraph).

Regarding claims 10 and 11, Nelson et al. teach reverse transcription of the polyA+ RNA isolated from normal human prostate tissue using oligo-dT-30 primer, reverse transcriptase, dATP, dTTP, dCTP, dGTP and $^{32}P$-dCTP, in a reaction at 42 °C for 30 minutes (page 210, fourth paragraph).

Regarding claim 13, Nelson et al. do not explicitly teach transformation of the members of non-normalized cDNA library into host cells, but since they do have clones, these could have been obtained only by transforming the library into host cells. See also Nelson et al. (Genomics, vol. 47, pp. 12-25, 1998; page 13, incorporated by reference).

Regarding claim 14, Nelson et al. teach growing host cells containing cDNA inserts on microtiter plates prior to hybridization (page 210, third paragraph).

B) Nelson et al. do not teach either the normalized or non-normalized libraries being full-length cDNA libraries.

C) Regarding claims 1 and 12, Frohman et al. teach a method (RACE) for obtaining full-length cDNA clones of low abundance mRNAs. The method involves amplification of 3' and 5' ends of cDNAs, followed by assembly of the fragments into a full-length cDNAs, which can then be cloned (Figure 1; page 8999, the last paragraph; page 9000).

It would have been *prima facie* obvious to one of ordinary skill in the art at the time of the invention to have used the libraries of full-length cDNAs of Frohman et al. in the method of library construction of Nelson et al. The motivation to do so would have been that using full-length cDNAs accelerated processes of gene characterization and protein expression.

***Response to Arguments***

8. Applicant's arguments filed on May 23, 2003 have been fully considered but they are not persuasive. Applicants argued rejections of claim 9 over Nelson et al. and Frohman et al., and since claim 9 has been incorporated into claim 1, these arguments are addressed here, because claim 1 is rejected over the combination of these two references.

With respect to claim 9, Applicants argue the following: RACE, which is a method to obtain full-length cDNAS, involves primers of known sequence, therefore it would not be useful for making full-length cDNA clones of a library with unknown sequences (page 10, third paragraph).

However, knowledge of the sequence of less than full-length cDNA clone is not required to use RACE to obtain full-length clones. As stated by Frohman et al. "It should also be possible to use modified RACE protocol to construct general cDNA libraries: one could reverse transcribe using $(dT)_{17}$ adaptor primer, tail the (-) strand products with G or C residues, generate a (+) strand with a different adaptor on its 5'end, and amplify the pooled cDNAs." (page 9002, the last

paragraph). Therefore, advance knowledge of the sequence of EST clones is not required to obtain full-length cDNAs.

9. Claim 6 is rejected under 35 U.S.C. 103(a) as being unpatentable over Nelson et al. in view of Frohman et al. (PNAS USA, vol. 85, pp. 8998-9002, 1988; cited in the previous office action), as applied to claims 1-5 above, and further in view of Somerville et al. (Science, vol. 285, p. 380-383, 1999).

A) Claim 6 is drawn to a plant cell being a soy, tobacco, wheat, rice or corn cell.

B) Nelson et al. and Frohman et al. teach construction of normalized full-length cDNA libraries from human prostate cells, but do not teach plant cells. However, Nelson et al. and Frohman et al. teach a method which is generally applicable to any type of cells and libraries constructed. Nelson et al. state that "...This procedure has several advantages over other methods such as normalization and subtraction for reducing the variation in abundance among the clones in a cDNA library. Standard library construction methods are employed without the necessity for PCR, reassociation reactions, or column purification of single-stranded DNA as used in several of the normalization and subtraction methods (citation omitted). Libraries previously made or purchased can be used without requiring new library construction." (page 214, second paragraph). In the Abstract, Nelson et al. point that "The identification of the entire complement of genes expressed in a cell, tissue or organism provides a framework for understanding biological properties and establishes a tool set for subsequent functional studies. The large-scale sequencing of randomly selected clones from cDNA libraries has been successfully employed as a method for identifying a large fraction of these expressed genes. However, this approach is limited by the inherent redundancy of cellular transcripts reflecting widely variant levels of gene transcription. As a result,

a high percentage of transcript duplications are encountered as the number of sequenced clones accrues."

C) Somerville et al. teach sequencing of Arabidopsis thaliana and rice genome. Rice was chose because of its similarities with wheat, maize and other cereals. They also teach that it is unlikely that other whole plant genomes would be sequenced because of the cost involved (page 380, third and fourth paragraphs).

It would have been *prima facie* obvious to one of ordinary skill in the art at the time of the invention to have applied the library normalization method of Nelson et al. and Frohman et al. to rice and other plant genomes of Somerville et al. The motivation to do so, expressly provided by Nelson et al., would have been that library normalization permitted full elucidation of genes expressed in a given cell.

10. Claim 8 is rejected under 35 U.S.C. 103(a) as being unpatentable over Nelson et al. in view of Frohman et al. (PNAS USA, vol. 85, pp. 8998-9002, 1988 ; cited in the previous office action), as applied to claims 1-5 and 7 above, and further in view of El-Meanawy et al. (Am. J. Physiol. Renal Physiol., vol. 279, p. F383-F392, 2000).

A) Claim 8 is drawn to a human cell being a kidney cell.

B) Nelson et al. and Frohman et al. teach construction of normalized cDNA libraries from human prostate cells, but does not teach human kidney cells. However, Nelson et al. teach a method which is generally applicable to any type of cells and libraries constructed. Nelson et al. state that "...This procedure has several advantages over other methods such as normalization and subtraction for reducing the variation in abundance among the clones in a cDNA library. Standard library construction methods are employed without the necessity for PCR, reassociation reactions, or column purification of single-stranded DNA as used in several of the normalization and

subtraction methods (citation omitted). Libraries previously made or purchased can be used without requiring new library construction."

C) El-Meanawy et al. teach construction of mouse kidney expression libraries using the SAGE (serial analysis of gene expression) method. The library construction was the first step to analysis of gene expression in progressive kidney disease based on mouse model (Abstract, page F383). However, they point to the fact that SAGE does not provide reliable detection of transcripts with low abundance (page F390, second paragraph).

It would have been *prima facie* obvious to one of ordinary skill in the art at the time of the invention to have used the library construction method of Nelson et al. and Frohman et al. to obtain clones from human kidney cell. The motivation to do so, expressly provided by El-Meanawy et al., would have been that expression libraries were a powerful tool to apply to elucidation of the mechanisms of renal disease because of the complexity of the disease and lack of effective treatments.

11. Claim 38 is rejected under 35 U.S.C. 103(a) as being unpatentable over Nelson et al. in view of Frohman et al. (PNAS USA, vol. 85, pp. 8998-9002, 1988; cited in the previous office action), as applied to claims 1 and 2 above, and further in view of Gress et al. (Mammalian Genome, vol. 3, pp. 609-619, 1992).

A) Claim 38 is drawn to the method of claim 2, wherein RNA sample is from a plurality of different cell types and/or tissue.

B) Neither Nelson et al. nor Frohman et al. teach RNA sample from a plurality of different cell types and/or tissue.

C) Gress et al. teach characterization of high-density cDNA library arrays by hybridization with labeled probes derived from cDNA pools of different tissues (Abstract). Gress et al. teach RNA samples from human fetal brain and Drosophila embryos (page 610, fourth paragraph).

It would have been *prima facie* obvious to one of ordinary skill in the art at the time of the invention to have used RNA samples and labeled probe libraries of Gress et al. in the library normalization method of Nelson et al. and Frohman et al. The motivation to do so, provided by Gress et al., would have been that using different types of tissues allowed selection of middle- to high abundance mRNAs (page 613, the last paragraph).

12. Claims 39, 40 and 41 are rejected under 35 U.S.C. 103(a) as being unpatentable over Nelson et al. in view of Frohman et al. (PNAS USA, vol. 85, pp. 8998-9002, 1988; cited in the previous office action), as applied to claims 1 and 2 above, and further in view of Xu et al. (Cancer Research, vol. 60, pp. 1677-1682, March 2000).

A) Claim 39 is drawn to the method of claim 2, wherein RNA sample is from a plurality of different cell individuals from the same species or organism. Claim 40 is drawn to the method of claim 1, wherein the labeled probe library is from another cell type or tissue of the same organism. Claim 41 is drawn to the method of claim 1, wherein the labeled probe library is from the same cell type or tissue from a different individual in the same organism.

B) Neither Nelson et al. nor Frohman et al. teach RNA sample from a plurality of different cell types and/or tissue, a labeled probe library is from the same cell type or tissue from a different individual of the same organism or a labeled probe library is from the same cell type or tissue from a different individual in the same organism.

C) Xu et al. teach normalization of prostate tumor and normal prostate tissue libraries (Abstract).

Regarding claim 39, Xu et al. teach a library generated from a pool of prostate tumor samples obtained from different clinical sources (page 1677, fourth paragraph; Table 1).

Regarding claim 40, Xu et al. teach labeled probe library from normal pancreas (page 1677, fifth paragraph; Table 1; page 1678, 6th paragraph).

Regarding claim 41, Xu et al. teach labeled probe library from normal prostate (Table 1; page 1678, 6th paragraph).

It would have been *prima facie* obvious to one of ordinary skill in the art at the time of the invention to have used the RNA samples from plurality of different individuals of Xu et al. in the combined library normalization method of Xu et al. The motivation to do so, provided by Xu et al., would have been that pooling resulted in identification of cancer-associated genes present in a small subpopulation of cancer patients (page 1681, 6th paragraph).

It would have been *prima facie* obvious to one of ordinary skill in the art at the time of the invention to have used the labeled probe library from another tissue type of Xu et al. in the combined library normalization method of Xu et al. The motivation to do so, provided by Xu et al., would have been that using RNA from different tissue type with similar function to the sample as a probe effectively eliminated genes shared by both tissues (page 1678, 6th paragraph).

It would have been *prima facie* obvious to one of ordinary skill in the art at the time of the invention to have used the labeled probe library from the same cell type of Xu et al. in the combined library normalization method of Xu et al. The motivation to do so would have been that using a probe from normal tissue hybridized to a sample of tumor tissue allowed elimination of non-specific clones.

13. Claims 15-19, 21, 23-31 and 44 are rejected under 35 U.S.C. 103(a) as being unpatentable over Nelson et al. (Genetic Analysis: Biomolecular Engineering, vol. 15, pp. 209-215, 1999; cited

in the IDS and in the previous office action) in view of Carninci et al. (Genome Res., vol. 10, pp. 1617-1630, October 2000; cited in the IDS).

A) Regarding claims 15 and 29, Nelson et al. teach construction of normalized cDNA library. First, a non-normalized cDNA library was constructed from polyA+ RNA isolated from normal human prostate tissue. Members of the library were separated by plating individual clones onto 384-well microtiter plates, grown and replica-spotted onto nylon membranes. The replicas were allowed to grow on the membranes, and then the colonies were lysed, providing DNA bound to the membrane (page 210, third paragraph). A labeled probe library was constructed by reverse transcription of the polyA+ RNA isolated from normal human prostate tissue using oligo-dT-30 primer, reverse transcriptase, dATP, dTTP, dCTP, dGTP and $^{32}$P-dCTP, in a reaction at 42 °C for 30 minutes (page 210, fourth paragraph).

The filters were hybridized with the labeled probe, exposed to phosphor capture screens and the signals were quantitated on a phosphorimager. Clones with signal intensities within the bottom quartile of the averaged intensities were selected (page 210, fifth and sixth paragraphs). A total of 842 cDNA clones were selected based on low hybridization intensities, of which 142 were discarded. Of the remaining 700 cDNA clones, 89% of sequences were genes of low abundance (= low expression) (page 211, fourth and fifth paragraphs; Table 1). The selected genes were pooled into a normalized library, PN001-NS (page 213, second paragraph; Table 1).

Regarding claims 16-19 and 21, Nelson et al. teach isolation of polyA+ RNA from normal human prostate cells. Nelson et al. do not specifically say that the RNA was mRNA, but only mRNA in the eucaryotic cells is polyadenylated (page 210, third paragraph).

Regarding claims 24 and 25, Nelson et al. teach reverse transcription of the polyA+ RNA isolated from normal human prostate tissue using oligo-dT-30 primer, reverse transcriptase, dATP,

dTTP, dCTP, dGTP and $^{32}P$-dCTP, in a reaction at 42 °C for 30 minutes (page 210, fourth paragraph).

Regarding claim 27, Nelson et al. do not explicitly teach transformation of the members of non-normalized cDNA library into host cells, but since they do have clones, these could have been obtained only by transforming the library into host cells. See also Nelson et al. (Genomics, vol. 47, pp. 12-25, 1998; page 13, incorporated by reference).

Regarding claim 28, Nelson et al. teach growing host cells containing cDNA inserts on microtiter plates prior to hybridization (page 210, third paragraph).

B) Nelson et al. do not teach: a) selecting sub-groups of members of the non-normalized cDNA library represented in higher amounts, b) either the normalized or non-normalized libraries being full-length cDNA libraries, c) constructing a labeled probe library from a sub-group of members, hybridizing labeled probe library to a group of members and identifying members which are not hybridized to the labeled probe library, and d) sequencing members of the cDNA library present in lower amounts and members of every subgroup, followed by pooling of all unique members. Nelson et al. teach that redundant clones can be pooled to comprise a secondary or tertiary generation probes for hybridization to array, and eliminate moderately abundant transcripts from further selection (page 214, second paragraph).

C) Regarding claim 15, Carninci et al. teach normalization of full-length cDNA libraries by hybridization of full-length cDNAs to biotinylated RNA, from which the cDNA has been obtained. The abundant cDNAs are removed by removing cDNA-RNA hybrids using magnetic beads with streptavidin. The resulting rare cDNAs are pooled (Fig. 1; page 1625, the last paragraph; page 1626, 1627; page 1628, paragraphs 1-6). The removed abundant cDNAs were stripped from the beads and re-used to prepare probe minilibraries (= selecting a first and subsequent groups of

members of the non-normalized cDNA library present in higher amounts) (page 1620, second and third paragraphs; page 1627, paragraphs 8 and 9; Table 1). For example, the normalized library 26-100 from the whole embryo was prepared by hybridization with total starting RNA, followed by two rounds of hybridization with mini-libraries: ms1, minilibrary of liver, lung, brain and placenta, and Nm1, RIKEN non-redundant minilibrary (Table 1). The members of the cDNA library present in lower amounts and the subgroup members were pooled to form normalized libraries (Table 1).

Regarding claims 23 and 26, Carninci et al. teach full-length non-normalized and normalized libraries (page 1618, third paragraph; page 1621, fourth paragraph).

Regarding claim 30, Carninci et al. teach preparation of labeled probe minilibraries from selected subsets of abundant cDNAs (page 1627, paragraphs 9-12; page 1628, first and second paragraphs).

Regarding claims 31 and 44, Carninci et al. teach sequencing of the members of the libraries prepared by normalization with starting RNA and subtraction with probe minilibraries (page 1620, fourth paragraph; Table 1).

It would have been *prima facie* obvious to one of ordinary skill in the art at the time of the invention to have combined the full-length cDNA library normalization method of Carninci et al. with a library normalization method of Nelson et al. The motivation to do so, provided by Carninci et al., would have been that the normalized cDNA library had a high percentage of full-length cDNAs which enhanced discovery of new genes (Abstract).

14. Claim 20 is rejected under 35 U.S.C. 103(a) as being unpatentable over Nelson et al. in view of Carninci et al. (Genome Res., vol. 10, pp. 1617-1630, October 2000; cited in the IDS), as applied to claims 15-19 above, and further in view of Somerville et al. (Science, vol. 285, p. 380-383, 1999).

A) Claim 20 is drawn to a plant cell being a soy, tobacco, wheat, rice or corn cell.

B) Nelson et al. and Carninci et al. teach construction of normalized full-length cDNA libraries from human prostate cells, but do not teach plant cells. However, Nelson et al. and Carninci et al. teach a method which is generally applicable to any type of cells and libraries constructed. Nelson et al. state that "...This procedure has several advantages over other methods such as normalization and subtraction for reducing the variation in abundance among the clones in a cDNA library. Standard library construction methods are employed without the necessity for PCR, reassociation reactions, or column purification of single-stranded DNA as used in several of the normalization and subtraction methods (citation omitted). Libraries previously made or purchased can be used without requiring new library construction." (page 214, second paragraph). In the Abstract, Nelson et al. point that "The identification of the entire complement of genes expressed in a cell, tissue or organism provides a framework for understanding biological properties and establishes a tool set for subsequent functional studies. The large-scale sequencing of randomly selected clones from cDNA libraries has been successfully employed as a method for identifying a large fraction of these expressed genes. However, this approach is limited by the inherent redundancy of cellular transcripts reflecting widely variant levels of gene transcription. As a result, a high percentage of transcript duplications are encountered as the number of sequenced clones accrues."

C) Somerville et al. teach sequencing of Arabidopsis thaliana and rice genome. Rice was chose because of its similarities with wheat, maize and other cereals. They also teach that it is unlikely that other whole plant genomes would be sequenced because of the cost involved (page 380, third and fourth paragraphs).

It would have been *prima facie* obvious to one of ordinary skill in the art at the time of the invention to have applied the library normalization method of Nelson et al. and Carninci et al. to rice and other plant genomes of Somerville et al. The motivation to do so, expressly provided by Nelson et al., would have been that library normalization permitted full elucidation of genes expressed in a given cell.

15. Claim 22 is rejected under 35 U.S.C. 103(a) as being unpatentable over Nelson et al. in view of Carninci et al. (Genome Res., vol. 10, pp. 1617-1630, October 2000; cited in the IDS), as applied to claims 15-19 and 21 above, and further in view of El-Meanawy et al. (Am. J. Physiol. Renal Physiol., vol. 279, p. F383-F392, 2000).

A) Claim 22 is drawn to a human cell being a kidney cell.

B) Nelson et al. and Carninci et al. teach construction of normalized cDNA libraries from human prostate cells, but does not teach human kidney cells. However, Nelson et al. and Carninci et al. teach a method which is generally applicable to any type of cells and libraries constructed. Nelson et al. state that "...This procedure has several advantages over other methods such as normalization and subtraction for reducing the variation in abundance among the clones in a cDNA library. Standard library construction methods are employed without the necessity for PCR, reassociation reactions, or column purification of single-stranded DNA as used in several of the normalization and subtraction methods (citation omitted). Libraries previously made or purchased can be used without requiring new library construction."

C) El-Meanawy et al. teach construction of mouse kidney expression libraries using the SAGE (serial analysis of gene expression) method. The library construction was the first step to analysis of gene expression in progressive kidney disease based on mouse model (Abstract, page

F383). However, they point to the fact that SAGE does not provide reliable detection of transcripts with low abundance (page F390, second paragraph).

It would have been *prima facie* obvious to one of ordinary skill in the art at the time of the invention to have used the library construction method of Nelson et al. and Carninci et al. to obtain clones from human kidney cell. The motivation to do so, expressly provided by El-Meanawy et al., would have been that expression libraries were a powerful tool to apply to elucidation of the mechanisms of renal disease because of the complexity of the disease and lack of effective treatments.

16. Claim 43 is rejected under 35 U.S.C. 103(a) as being unpatentable over Nelson et al. in view of Carninci et al. (Genome Res., vol. 10, pp. 1617-1630, October 2000; cited in the IDS).

A) Claim 43 is drawn to the method of claim 15, further comprising repeating steps d-f with every subgroup of the non-normalized cDNA library represented in higher amounts.

B) Neither Nelson et al. nor Carninci et al. specifically teach repeating the process of group selection. However, Nelson et al. suggests pooling high abundance cDNAs to comprise a secondary or tertiary generation probes for hybridization to array, and eliminate moderately abundant transcripts from further selection (page 214, second paragraph).

It would have been *prima facie* obvious to one of ordinary skill in the art at the time of the invention to have used repeated selection and subtraction of sub-groups of Carninci et al. to generate normalized cDNA library. The motivation to do so, provided by Nelson et al., would have been that repeated subtraction with second and third generation probes eliminated moderately abundant transcripts (page 214, second paragraph).

17. Claim 45 is rejected under 35 U.S.C. 103(a) as being unpatentable over Nelson et al. in view of Carninci et al. (Genome Res., vol. 10, pp. 1617-1630, October 2000; cited in the IDS).

A) Claim 45 is drawn to the method of claim 44 where every unique member is pooled.

B) Neither Nelson et al. nor Carninci et al. specifically teach pooling of unique members of the sequenced members of the non-normalized cDNA library present in lower amounts and of the members in sub-groups.

C) Carninci et al. teach sequencing of the normalized libraries, which consist of the the non-normalized cDNA library present in lower amounts and of the members in sub-groups. They teach that each normalized library had unique members (Table 1; page 1620, paragraph 4).

It would have been prima facie obvious to one of ordinary skill in the art at the time of the invention to have pooled the unique members of the sequenced libraries of Carninci et al. The motivation to do so, provided by Carninci et al., would have been that the unique libraries increased recovery of unknown genes (page 1620, the last sentence; page 1621, first paragraph).

18. Claims 32, 37 and 46 are rejected under 35 U.S.C. 103(a) as being unpatentable over Nelson et al. (Genetic Analysis: Biomolecular Engineering, vol. 15, pp. 209-215, 1999; cited in the IDS and in the previous office action) in view of Carninci et al. (Genome Res., vol. 10, pp. 1617-1630, October 2000; cited in the IDS).

A) Regarding claim 32, Nelson et al. teach construction of normalized cDNA library. First, a non-normalized cDNA library was constructed from polyA+ RNA isolated from normal human prostate tissue. Members of the library were separated by plating individual clones onto 384-well microtiter plates, grown and replica-spotted onto nylon membranes. The replicas were allowed to grow on the membranes, and then the colonies were lysed, providing DNA bound to the membrane (page 210, third paragraph). A labeled probe library was constructed by reverse transcription of the polyA+ RNA isolated from normal human prostate tissue using oligo-dT-30 primer, reverse

transcriptase, dATP, dTTP, dCTP, dGTP and $^{32}$P-dCTP, in a reaction at 42 °C for 30 minutes (page 210, fourth paragraph).

The filters were hybridized with the labeled probe, exposed to phosphor capture screens and the signals were quantitated on a phosphorimager. Clones with signal intensities within the bottom quartile of the averaged intensities were selected (page 210, fifth and sixth paragraphs). A total of 842 cDNA clones were selected based on low hybridization intensities, of which 142 were discarded. Of the remaining 700 cDNA clones, 89% of sequences were genes of low abundance (= low expression) (page 211, fourth and fifth paragraphs; Table 1). The selected genes were pooled into a normalized library, PN001-NS (page 213, second paragraph; Table 1).

B) Nelson et al. do not teach RNA sample from a plurality of different tissues, developmental stages or individuals from the same species of organism, RNA from substantially every cell type and/or tissue from the same species of organism or RNA from different developmental stages of the same type of tissue.

C) Regarding claims 32 and 37, Carninci et al. teach preparation of normalized cDNA libraries from RNA samples containing plurality of different tissues and RNA from substantially every cell type and/or tissue from the same species of organism, such as whole embryos (Table 1, library 26-000).

Regarding claim 46, Carninci et al. teach preparation of normalized cDNA libraries from different developmental stages of the same type of tissue, namely, adult testis and embryo testis (Table 1, libraries 49-304 and 60-304).

It would have been *prima facie* obvious to one of ordinary skill in the art at the time of the invention to have used RNA sample of Carninci et al. in the library normalization method of Nelson et al. The motivation to do so would have been that libraries with cDNAs from all tissue types were

used to estimate abundance of all tissue-specific transcripts, and libraries with cDNAs from different developmental stages of the same tissue were used to monitor development-related patterns of gene expression.

19. No claims are allowed.

### *Conclusion*

20. Applicant's amendment necessitated the new ground(s) of rejection presented in this Office action. Accordingly, **THIS ACTION IS MADE FINAL**. See MPEP § 706.07(a). Applicant is reminded of the extension of time policy as set forth in 37 CFR 1.136(a).

A shortened statutory period for reply to this final action is set to expire THREE MONTHS from the mailing date of this action. In the event a first reply is filed within TWO MONTHS of the mailing date of this final action and the advisory action is not mailed until after the end of the THREE-MONTH shortened statutory period, then the shortened statutory period will expire on the date the advisory action is mailed, and any extension fee pursuant to 37 CFR 1.136(a) will be calculated from the mailing date of the advisory action. In no event, however, will the statutory period for reply expire later than SIX MONTHS from the date of this final action.

Any inquiry concerning this communication or earlier communications from the examiner should be directed to Teresa E Strzelecka whose telephone number is (703) 306-5877. The examiner can normally be reached on M-F (8:30-5:30).

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Gary Benzion can be reached at (703) 308-1119. The fax phone number for the organization where this application or proceeding is assigned is (703) 872-9306.

Any inquiry of a general nature or relating to the status of this application or proceeding should be directed to the receptionist whose telephone number is (703) 308-0196.

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8/20/03

KENNETH R. HORLICK, PH.D
PRIMARY EXAMINER
8/21/03